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Short Communication

Comparative study of the essential oil composition and antimicrobial activity of *Leonotis leonurus* and *L. ocymifolia* in the Eastern Cape, South Africa

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Species of *Leonotis* are among the frequently-used herbal remedies to treat various bronchial illness and epilepsy. The essential oils of the leaf and flower of *L. leonurus* and *L. ocymifolia* growing in the Eastern Cape of South Africa were analysed by GC-MS. Major constituents of the *L. leonurus* oils were limonene (7.2–15.6%), (Z)-β-ocimene (7.5–10.8%), γ-terpinene (4.0–4.7%), β-caryophyllene (15.2–19.6%), α-humulene (4.6–6.5%) and germacrene D (18.9–20.0%), while the essential oils of *L. ocymifolia* had (Z)-β-ocimene (13.0–15.2%), nonanal (5.5%) β-caryophyllene (21.4–30.8%), α-humulene (9.1–11.6%), germacrene D (21.5–21.7%) and T-muurolol (4.6%) as the prominent compounds. The oils exhibited a broad spectrum antibacterial activity against Gram-positive (*Bacillus subtilis*, *Bacillus cereus*, *Micrococcus kiristinae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella sonnei*) bacteria with MIC values ranging from 1.25–0.039mg ml^{-1}.

Species of *Leonotis* are widely distributed in Africa where they have a long history of use for medicinal purposes. In South Africa, many members of the genus are found in the southwest, spreading to the Eastern Cape (Watt and Breyer-Brandwijk 1962, Batten 1988). In the Eastern Cape Province, some of the species are used for the treatment of coughs, chest troubles, bronchial asthma, stomach aches, skin diseases, haemorrhoids and epilepsy (Watt and Breyer-Brandwijk 1962, Batten and Bokelmann 1966, Gledhill 1969, Batten 1988).

Leonotis leonurus (L.) R. Br. and *Leonotis ocymifolia* (Burm.f.) Iwarsson are the two most commonly-used species in the Province. *L. ocymifolia* grows mostly along the coastal region and is particularly used for the treatment of nerve weakness, while *L. leonurus* grows in moist areas, along hill-slopes and is commonly used for the treatment of liver inflammation (Watt and Breyer-Brandwijk 1962, Batten and Bokelmann 1966, Gledhill 1969, Van Wyk *et al.* 1997, Pienaar 1984, Batten 1988, Batten *et al.* 2001).

Previous essential oil analyses of *Leonotis* species from Nigeria, South India and Rwanda have been reported. The Nigerian and Rwanda types were dominated by sesquiterpenoids while South India had phenols and monoterpenoids as the major components, indicating a chemotypic difference (Thoppil and Jose 1995, Muhayimana *et al.* 1998, Oyedeji *et al.* 1999). *L. leonurus* flower oil from Portugal had α-pinene, limonene, β-caryophyllene, α-humulene and caryophyllene oxide as the major components (Pedro *et al.* 1991).

These two important medicinal plants growing in South Africa are highly aromatic, yet, to the best of our knowledge, there is no information on the type and constituents of their volatiles. In this paper, we present a comparative analysis of the oils found in *L. leonurus* and *L. ocymifolia* in South Africa with their antimicrobial activity.

Fresh plants of *L. leonurus* and *L. ocymifolia* were collected from the wild around the University of Fort Hare campus, and voucher specimens were deposited at the University Herbarium.

The fresh plant materials were carefully separated into flowers and leaves. About 400g of the leaves and 200g of the flowers of each plant were hydrodistilled separately in an all-glass Clevenger-type apparatus in accordance with the British Pharmacopoeia (BP) (1980) method, for 3h.

GC-MS analyses of the oils were performed on a Hewlett Packard Gas Chromatograph HP 5973 interfaced with a VG analytical 70-280s double-focussing mass spectrometer. Electron ionisation was at 70eV with an ion-source temperature at 240°C. HB-5 column was used (30m x 0.25mm id) similar to DB 5, film thickness was 0.25μm, while

helium was used as the carrier gas. The oven temperature was 70–240°C at 5°C min^{-1}. Oil (0.2µl) was injected into the GCMS. n-Alkanes were run under the same conditions for the retention indices determination.

Identification of the oil constituents was accomplished by comparison of their mass spectra and retention indices with those of standard samples and literature (Adams 1989, Joulain and Koenig 1998).

A collection of eight laboratory bacteria which included five Gram-positive and three Gram-negative strains was obtained from the Microbiology Department, Rhodes University. These are *Bacillus subtilis*, *Bacillus cereus, Micrococcus kiristinae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Shigella sonnei*. The MIC values of the essential oil were determined with a microplate dilution method against the bacteria using 96-well microtitre plates. Essential oil in 40µl of hexane was dissolved in 40µl of acetone. Each test organism was prepared by diluting 24h-old broth culture with sterile nutrient broth. The culture was then diluted 100-fold to give approximately 10^6 bacteria ml^{-1}. The microtitre plates were prepared using serial dilution (Afolayan and Meyer 1997, Amvam Zollo *et al.* 1998, Eloff 1998) and incubated for 24–48h at 37°C. As an indicator of bacterial growth, 40µl of 0.2mg ml^{-1} p-iodonitrotetrazolium (INT) solution was added to each well and incubated at 37°C for 30min. The colourless tetrazolium salt was reduced to a red-coloured product by biological activity of the organisms, thereby making the inhibition of bacterial growth visible as clear wells. MIC values were recorded as the lowest concentration resulting in complete inhibition of bacterial growth. Each treatment was replicated three times. Streptomycin, chloramphenicol, solvents and sample-free solutions were used as positive and negative controls.

A light yellow oil with 0.03% yield was distilled from the leaves of *L. leonurus*, while a colourless, pleasant-scented oil was obtained from its flowers at 0.05% yield. A total of 40 compounds were found from the GCMS analysis of the leaves of *L. leonurus*; 30 of these were identified, which constituted 96.8% of the oil composition (Table 1), whereas 31 compounds out of 38 (representing 96.3%) were identified in the essential oil of the flower. Germacrene D (18.9%), limonene (15.6%), β-caryophyllene (15.2%), (Z)-β-ocimene (7.5%), γ-terpinene (4.7%), p-cymene (3.5%), terpinolene (3.1%), α-humulene (4.6%), (E)-β-ocimene (3.0%) and bicyclogermacrene (3.0%) were the major components of the leaf oil. The flowers, on the other hand, had germacrene D (20.0%), β-caryophyllene (19.6%), (Z)-β-ocimene (10.8%), limonene (7.2%), α-humulene (6.5%), γ-terpinene (4.0%) and bicyclogermacrene (3.6%) as the prominent constituents.

Colourless essential oils were obtained from the leaves and flowers of *L. ocymifolia* with each yielding 0.06%. The chemical profile was similar to that of *L. leonurus* with sesquiterpenoid dominating the oil composition (65.9–78.9%). β-caryophyllene was the main constituent (21.4–30.8%) of the *L. ocymifolia* oils; this was closely followed by germacrene D (21.5–21.7%), (Z)-β-ocimene (13.0–15.2%) and α-humulene (9.1–11.6%). Bicyclogermacrene (4.0%) was found in an appreciable amount in the leaf oil while T-muurolol (4.6%) was present in the flower extract. It was interesting to note the variance in the major monoterpenoid constituents of the oils between the leaves and flowers of *L. ocymifolia*. (Z)-β-ocimene and (E)-β-ocimene were the two main compounds in the leaf oil (13.0 and 3.5% respectively) while the flower oil had (E)-β-ocimene (15.2%) and nonanal (5.5%) as dominating monoterpenoids.

Again, compositional difference was observed in the two species. In the leaf and flower oils of *L. leonurus*, limonene occurred at higher concentrations (15.6% and 7.2%, respectively), while it was present in a trace amount in the

Table 1: Percentage composition of the leaf and flower oils of *L. leonurus* and *L. ocymifolia*

		L. leonurus		*L. ocymifolia*	
Compound	RI[a]	Leaf	Flower	Leaf	Flower
α-phellandrene	1 003	0.7	0.3	0.2	0.6
α-terpinene	1 015	0.2	0.1	–	–
p-cymene	1 025	3.5	1.2	–	1.5
limonene	1 032	15.6	7.2	1.9	t
(Z)-β-ocimene	1 040	7.5	10.8	13.0	15.2
(E)-β-ocimene	1 046	3.0	2.5	3.5	0.7
γ-terpinene	1 060	4.7	4.0	–	–
terpinolene	1 089	3.6	2.4	–	–
linalool	1 099	0.5	0.3	–	1.4
nonanal	1 105	–	–	–	5.5
allo-ocimene*	1 120		0.2	0.5	t
terpinen-1-ol	1 145	0.7	–	0.4	–
terpinen-4-ol	1 179	–	0.3	–	–
α-terpineol	1 192	–	0.3	–	–
decanal	1 208	–	–	–	2.3
α-cubebene	1 351	1.5	1.4	–	–
α-copaene	1 382	1.4	1.4	1.6	2.0
β-bourbonene	1 394	2.1	2.0	1.6	t
β-cubebene	1 395	2.2	2.4	–	t
β-elemene	1 398			0.7	t
β-caryophyllene	1 428	15.2	19.6	30.8	21.4
β-gurjunene	1 432	0.5	0.5	0.4	t
β-copaene	1 446	0.1	–	–	–
guaia-3,7-diene	1 448	–	–	0.8	1.1
α-humulene	1 461	4.6	6.5	11.6	9.1
germacrene D	1 484	18.9	20.0	21.5	21.7
bicyclogermacrene	1 495	3.0	3.6	1.8	1.5
germacrene A	1 501	0.2	0.2	–	–
γ-cadinene	1 520	1.6	2.0	1.0	1.9
δ-cadinene	1 524	1.5	1.5	1.2	1.8
cadina-1,4-diene	1 532	0.3	0.3	–	–
spathulenol	1 577	0.9	0.8	–	–
caryophyllene oxide	1 582	2.5	2.9	4.0	0.9
10-*epi*-globulol	1 585	0.2	0.4	–	–
humulene epoxide*	1 592	0.5	0.5	0.7	t
cubenol	1 642	0.2	0.2	–	–
T-muurolol	1 645	0.2	0.2	1.2	4.6
α-cadinol	1 660	–	0.6	–	t
Total (%)		96.8	96.3	98.5	93.1
Unknown (%)		3.2	3.7	1.5	6.7

t = trace amount <0.01

[a] = retention index on an HB-5 capillary column at 5°C min^{-1} from 70–240°C

* = correct isomer not identified

Table 2: Antibacterial activity of the essential oils of *L. leonurus* and *L. ocymifolia*

	Minimum Inhibitory Concentration (mg ml^{-1})							
	L. leonurus		*L. ocymifolia*		Streptomycin	Chloramphenicol	Hexane	Acetone
Bacterial species	F	L	F	L				
Gram +ve								
Bacillus cereus	0.078	0.078	0.156	0.156	2.500	0.039	–	–
Bacillus subtilis	0.078	0.078	1.250	1.250	2.500	0.039	–	–
Micrococcus kristinae	0.156	0.156	0.156	0.156	0.039	0.039	–	–
Staphylococcus epidermidis	0.625	0.039	0.625	0.625	0.039	2.500	–	–
Staphylococcus aureus	0.313	0.313	0.313	0.156	0.039	1.250	–	–
Gram –ve								
Escherichia coli	0.156	0.156	0.625	0.625	0.039	0.039	–	–
Pseudomonas aeruginosa	1.250	1.250	0.313	0.313	0.039	0.039	–	–
Shigella sonnei	0.313	0.156	0.313	0.156	0.039	0.039	–	–

F = flower oil; L = leaf oil; – no inhibition of growth

flowers and 1.93% in the leaves of *L. ocymifolia* essential oils. Also, β-caryophyllene and germacrene D were found at higher concentrations in the essential oils of *L. ocymifolia*, making the plant a good source of β-caryophyllene, α-humulene, germacrene D and (Z)-β-ocimene while limonene could be readily obtained from *L. leonurus*. *P*-cymene was considerably lower (1.2–1.5%) in the two flower oils. This compound was not detected in the leaf oil of *L. ocymifolia* but was present in an appreciable amount (3.5%) in the leaf oil of *L. leonurus*. Furthermore, the concentration of T-muurolol was fairly high in the flower oil of *L. ocymifolia* when compared to the remaining three oils. Nonanal was substantially present in the essential oil of *L. ocymifolia* flowers but not detected in the leaf oil and the flowers of *L. leonurus*. γ-Terpinene and terpinolene were not detected in the oils of *L. ocymifolia*. Generally, *L. ocymifolia* had a higher oil yield (0.06%) when compared to *L. leonurus*.

Information on the oil constituents found in the species of *Leonotis* may be significant in the chemotaxonomy of the group. This type of information is also important in our understanding of the medicinal use of the group.

The antibacterial test also reveals both oils having a broad spectrum antimicrobial activity against all the tested organisms with MIC ranging from 0.039–1.25mg ml^{-1} (Table 2). The oil was however more active against the Gram-positive bacteria than the Gram-negative ones. It is interesting to note that while streptomycin was only effective against *B. cereus and B. subtilis* bacteria at 2.5mg ml^{-1}, the essential oil of this plant inhibited the growth of these organisms at concentrations of 0.078–1.250mg ml^{-1}.

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